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Review Article: Plague Gives Surprises in the First Decade of the 21st Century in the United States and Worldwide

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Abstract. Plague is an ancient disease caused by the bacterium *Yersinia pestis* and transmitted by rodent flea bites that continues to surprise us with first-ever events. This review documents plague in human cases in the 1st decade of the 21st century and updates our knowledge of clinical manifestations, transmission during outbreaks, diagnostic testing, antimicrobial treatment, and vaccine development. In the United States, 57 persons were reported to have the disease, of which seven died. Worldwide, 21,725 persons were affected with 1,612 deaths, for a case-fatality rate of 7.4%. The Congo reported more cases than any other country, including two large outbreaks of pneumonic plague, surpassing Madagascar, which had the most cases in the previous decade. Two United States scientists suffered fatal accidental exposures: a wildlife biologist, who carried out an autopsy on a mountain lion in Arizona in 2007, and a geneticist with subclinical hemochromatosis in Chicago, who was handling an avirulent strain of *Y. pestis* in 2009. Antimicrobial drugs given early after the onset of symptoms prevented many deaths; those recommended for treatment and prophylaxis included gentamicin, doxycycline, and fluoroquinolones, although fluoroquinolones have not been adequately tested in humans. Fleas that do not have their guts blocked by clotted blood meals were shown to be better transmitters of plague than blocked fleas. Under development for protection against bioterrorist use, a subunit vaccine containing F1 and V antigens of *Y. pestis* was administered to human volunteers eliciting antibodies without any serious side effects. These events, although showing progress, suggest that plague will persist in rodent reservoirs mostly in African countries burdened by poverty and civil unrest, causing death when patients fail to receive prompt antimicrobial treatment.

INTRODUCTION

As a zoonotic, pandemic scourge, plague has killed millions of persons during three pandemic waves from Biblical times to the present but has been slowed in the past century by improvements in rodent-proof housing, urban hygiene, and clothing that protects against flea bites.[1] The causative agent *Yersinia pestis* is a Gram-negative bacterium that harbors three virulence plasmids—pFra that encodes the anti-phagocytic capsular protein fraction 1 (F1) and the murine toxin that enables bacteria to survive in the flea gut; pYV that encodes V antigen and *Yersinia* outer proteins (Yops), which disrupt phagocytosis and reduce inflammation; and pPla that makes a plasminogen activator that allows bacteria to spread in tissues by dissolving fibrin clots.[2] Reservoirs in nature are diverse rodent species, including rats, field mice, gerbils, jirds, and marmots, which transmit infections to one another by flea bites, with humans becoming accidental hosts when they contact rodent fleas or handle infected animals. The predominant clinical presentation is bubonic plague, in which an enlarged lymph node called a bubo develops most often in the femoral or inguinal area. Pneumonic plague is a rare form of pneumonia that is transmitted from coughing persons by aerosol droplets. Human mortality rates have decreased with the use of antimicrobial drugs during the past six decades, but these drugs cannot control infection in rodent reservoirs. Vaccines have been used and new ones are in development, but they can only, at best, prevent infections in people exposed to infected fleas or aerosols from coughing patients or created by bioterrorists. The purpose of this review is to examine the importance of plague during the first decade of this century with an emphasis on novel events and progress of clinical knowledge (Table 1).[3–19] Relevant literature was obtained by searching Pubmed for papers covering the years 2000–2009.

CLINICAL FEATURES

The majority of reported plague cases are bubonic, the only distinctive presentation often recognizable by patients and physicians in endemic areas.[16,20] In 671 reported cases in Madagascar in 2000–2001, 96% were bubonic and 3% pneumonic.[13] After an incubation period of ~2–8 days following a bite of an infected rodent flea, unnoticed by patients and during which bacteria migrate to a regional lymph node, patients note the sudden onset of fever and chills accompanied by a painful swelling of an inflamed lymph node, the bubo. Most buboes are in the femoral and inguinal regions caused by flea bites on the legs. Bites on the hands and arms, or direct contact from butchering or skinning an animal, result in an axillary bubo, whereas bites on the head and neck give rise to cervical buboes. Buboes enlarge rapidly, capable of reaching the size of an egg in 1–2 days, and are tender, warm to touch, and sometimes show surrounding edema with erythema of overlying skin. When buboes have been examined histologically, they show destruction of lymph node architecture, hemorrhagic necrosis, neutrophils, and massive numbers of extracellular bacteria. In a rat model of experimental bubonic plague, lymph nodes contained fewer neutrophils at 36 hours after infection than in animals infected with an avirulent strain of *Y. pestis* lacking the pYV plasmid, suggesting that virulence depends on inhibition of neutrophil activity in the bubo,[21] a view consistent with other studies of immune mechanisms that allow *Y. pestis* to escape killing by cells of the immune system.[22] Bacteria disseminate from buboes into the blood to the spleen, liver, bone marrow, and other organs. In about 50% of untreated patients, bacteria grow rapidly to achieve high-grade

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TABLE 1
Unique plague events in 2000–2009

Event	Year of event or (report)	Place	References
Outbreaks of pneumonic plague in miners in diamond and gold mines	2005 and 2006	Oriental Province of Congo	3,4
Outbreak of pneumonic plague originating from a sick dog	2006	Qinhai Province of China	5
Re-emergence of disease after decades-long absences	2003, 2008, and 2009	Algeria, Libya	6,7,8
Outbreak of pharyngeal plague traced to meat of a camel	1997 (2008)	Jordan	9
Outbreak of gastroenteritis traced to meat of a sick camel	2007	Afghanistan	10
Fatal pneumonic plague after a biologist did an autopsy on a mountain lion	2007	Arizona	11
Fatal septicemic plague in a scientist who handled cultures of an attenuated strain of *Y. pestis*	2009	Chicago	12
Deployment of rapid antigen capture dipstick for diagnosis	Entire decade	Madagascar and other African countries	13
Randomized clinical trial comparing gentamicin and doxycycline	2002	Tanzania	14
Recommendation of ciprofloxacin for treatment and prophylaxis of pneumonic plague	2004	United States	15,16
Discovery that unblocked fleas are more efficient transmitters than blocked fleas	2006	United States	17
Phase II and III vaccine trials using recombinant antigens F1 and V of *Y. pestis*	(2005, 2009)	United Kingdom	18,19

bacteremia, causing death from septic shock about 3–6 days after the onset of symptoms. The second most common clinical form is septicemic plague, occurring in about one-third of cases in the United States,[23,24] in which bacteria inoculated by a flea bite bypass the lymph nodes and grow in the blood. Septicemic plague is rarely diagnosed outside the United States because blood cultures are not routinely obtained in endemic countries for diagnosis in febrile patients. The third most common form is plague pneumonia, which develops either as secondary pneumonia, after a patient with bubonic or septicemic illness gets bacteria from the blood into his lungs, or, which as primary pneumonia, is acquired by inhalation of an infected aerosol generated by a coughing patient or from an infected animal. Symptoms in primary pneumonia are not unique for plague but are likely to include cough, severe dyspnea, and hemoptysis with rapid progression in untreated patients to death in about 3 days after the onset of symptoms. Humans are not a reservoir for sustained person-to-person transmission because they either die quickly or survive after prompt treatment that kills their bacteria, and they transmit infection ineffectively to small numbers of close contacts. Septicemic plague has a case-fatality rate of about 50%, which is higher than in bubonic plague caused by delays in making a correct diagnosis. Pneumonic plague is fatal in all instances unless antimicrobial treatment is started on the first day of symptoms.

The skin may show pustules, ulcers, eschars, or carbuncles at the presumed site of the flea bite, but most patients have normal skin. In the later stages of severe disease, some patients develop petechiae and purpura caused by vasculitis and disseminated intravascular coagulation, leading rarely to gangrene and a need to amputate fingers and toes.[25]

A rare clinical form reported during the decade is pharyngeal plague, which presents as a sore throat with cervical lymphadenopathy after ingesting or inhaling bacteria. An outbreak in Jordan in 1997 affected 12 persons who had eaten raw or cooked camel meat from the same camel 2–4 days before the onset of symptoms[9]; although one developed pneumonia and two underwent appendectomies, all survived after gentamicin treatment. Another outbreak, the first-ever of plague in Afghanistan, was linked to eating or handling camel meat from a slaughtered sick animal causing fever and gastroenteritis in 83 persons, of whom 17 died.[10] Camels in these instances were assumed to be infected by rodent flea bites.

REPORTED OCCURRENCE WORLDWIDE

Patients with plague were reported to the World Health Organization (WHO) from 16 countries, with African countries responsible for more than 97% of the world's 21,725 cases.[26–28] These numbers were based on clinical and epidemiological information available to health departments, but laboratory confirmation by cultures or serology or antigen detection was not available for most of them. Deaths were recorded in 1,612 patients, for a case-fatality rate of 7.4%. The Congo was the country with the most cases (Table 2), and all these occurred in the Oriental Province following years of civil strife and influxes of displaced persons. The Ituri focus in this province is considered the most active worldwide, accounting for more than 1,000 cases each year.[28] Madagascar had the second largest number of cases, after having been the leading country for plague occurrence in the previous decade. The United States reported only 56 cases, with seven deaths, but was noteworthy for reporting cases in every year of the decade. These patients acquired their infections in New Mexico, Arizona, Colorado, California, and Texas. In 2010, 2 cases occurred in Oregon, which had been free of the disease since 1995.[29] The ascendancy of Congo's cases has been attributed to changes in human behavior caused by civil wars and deterioration of health services, also perhaps to increased contacts of humans with rodents and their fleas.[30] Additionally, two large outbreaks of pneumonic plague occurred in the Congo in 2005 and 2006.[3,4] The first was in Zobia in the Oriental Province affecting 130 men working in a diamond mine, of whom 57 died. Diagnoses were established by serology. The second outbreak was at a gold mine near Bolebole about 200 km from Zobia affecting 162 persons with 45 deaths, from whom four sputum specimens yielded *Y. pestis* in cultures. An outbreak of suspected pneumonic plague affected 12 persons, of whom 11 died, in the Nebbi District of Uganda in 2006.[27] Another outbreak of pneumonic plague occurred in Qinghai Province of China (north of Tibet) in 2009 affecting 12 persons, of whom three died.[5] The index case was a herdsman exposed to a sick

TABLE 2

Reports of human plague in countries with more than 40 cases in the 1st decade of the 21st century in the order from greatest to least numbers of reported patients*

Country	Years 2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	Total cases for decade
Congo	371	509	798	1,092	1,042	1,434	1,789	966	1,962	618	10,581
Madagascar	1,333	804	658	933	1,214	421	412	583	535	289	7,182
Zambia	0	850	0	0	0	0	0	425	34	0	1,309
Uganda	202	319	60	24	0	0	24†	277	40	26	972
Mozambique	451	73	45	31	0	0	0	0	0	0	600
Tanzania	74	2	19‡	0	0	0	0	59	74	2	230
China	25	79	68	13	21	5	0	2	2	12	227
Peru	17	12	9	19	8	16	25	26	28	25	185
Malawi	78	0	92	0	0	0	0	0	0	0	170
Indonesia	0	1	1	2	7	11	4	71	3	0	100
United States	6	2	2	1	3	8	17	7	3	8	57
Vietnam	22	13	8	0	0	0	0	0	0	0	43

*Data from World Health Organization.[26]
†Investigation by Uganda Ministry of Health and CDC found 127 cases.[27]
‡An antibiotic trial in Tanzania made laboratory diagnoses in 63 patients.[14]

dog. Both the index case and the dog died. Other cases were contacts of the index case, including a doctor. *Y. pestis* was isolated from two dogs and five of the patients. This outbreak was remarkable because it is the first pneumonic plague outbreak originating from a sick dog and because dogs are considered naturally resistant to the disease despite ingesting infected rodents in endemic areas. Previous outbreaks have been linked to sick cats[31] or to humans with bubonic plague who developed secondary pneumonia. Plague re-emerged in Algeria in 2003 with 18 cases and one death after an absence of any cases for 57 years.[6] At a different rural focus in Algeria in 2008, four nomads acquired plague, resulting in one death from pneumonia.[7] The disease re-emerged also in Libya in 2009, affecting five persons with one fatality, after an absence of reported cases for 25 years.[8]

DEATHS OF TWO UNITED STATES SCIENTISTS IN ACCIDENTAL EXPOSURES

A 37-year-old male wildlife biologist working at Grand Canyon National Park in Arizona performed an autopsy on a mountain lion that he had tracked by a radio collar and found dead in 2007. Three days later he developed fever and cough productive of blood-tinged sputum. Four days after the onset of symptoms, he was found dead at his home. An autopsy revealed plague pneumonia with identification of *Y. pestis* from organ specimens. The biologist's autopsy of the mountain lion showed blood in the nares, chest cavity, and lung, as well as *Y. pestis* in tissue specimens.[11] The patient had thought the animal died of chest trauma inflicted by another lion, explaining why he took no precautions while handling the carcass and not wearing gloves or face mask during the autopsy carried out in his garage.

The second death occurred in 2009, when a 60-year-old male geneticist in Chicago was using plague bacteria for gene insertions and transposon mutagenesis. He handled an avirulent mutant strain lacking chromosomal genes for iron acquisition and pigment formation when grown on Congo red agar, designated as non-pigmented KIM 27.[12] This strain had been attenuated by serially passaging the virulent strain KIM 10+, which had been originally isolated from a patient with plague pneumonia in Iran in 1961.[32] As this attenuated bacterium was exempt from select agent registration, he was known not to adhere to recommended precautions of wearing gloves when handling it. He was an insulin-dependent diabetic.[33,34] After 6 days of symptoms of fever, shortness of breath, dry cough, and weakness, he was admitted to the hospital. His temperature was 38.3°C, pulse 106/minute, respirations 42/minute, and blood pressure 106/75 mm Hg. The white blood cell count was 79,200/mm^3, and bacteria were visible in a blood smear. He was intubated, resuscitated, but died 12 hours after admission. Blood cultures yielded growth of *Y. pestis*. Iron deposits were seen in the liver, consistent with hereditary hemochromatosis, which was confirmed by iron measurements and genetic testing. Examination of *Y. pestis* from the blood cultures showed it to be still non-pigmented as well as non-lethal when injected into laboratory mice. Thus, there was no mutational reversion of the avirulent bacteria to virulence. It was speculated that his hemochromatosis provided a source of iron that the mutant bacteria could not capture in normally low body amounts for rapid growth.[32,33] Support for this mechanism was provided by experiments in a genetically defined strain of mice that mimics human hemochromatosis, showing that the nonpigmented *Y. pestis* in this case exhibited greater lethality in mice with hemochromatosis than in normal mice.[35]

Both of these scientists sought medical care for their symptoms but neither told doctors about suspected exposures, leading to missed diagnoses and omission of needed antibiotic treatment. Our lessons should be a reminder that handling dead animals in a plague-endemic area is dangerous and that avirulent mutants of *Y. pestis* can unexpectedly cause disease in immunocompromised persons, specifically those with hemochromatosis and diabetes mellitus, who do not adhere to laboratory safety precautions.

DIAGNOSIS

A diagnosis is best established by culturing *Y. pestis* from a bubo aspirate, blood, or sputum. Laboratories use routine media, such as blood agar, McConkey agar, and trypticase soy broth, followed by biochemical tests to identify the organism. Hospital laboratories are prone to misidentify isolates as *Proteus* species or other Enterobacteriaceae.[24] Reference laboratories use fluorescent antibodies to show bacteria in specimens, specific bacteriolytic phage, antigen detection, serologic diagnosis by detecting antibodies against F1 antigen

in acute and convalescent sera, and molecular techniques using genetic probes in polymerase chain reactions (PCR). A preliminary diagnosis can be made often by microscopic examination of the bubo aspirate or sputum specimen stained with Gram stain or methylene blue-containing Wayson's stain that reveals characteristic Gram-negative bacilli and bipolar morphology.

A rapid diagnostic test using a dipstick employing monoclonal capture antibodies against F1 antigen for use in the field was developed by the Pasteur Institute in Madagascar and is available in African countries.[13,36,37] With high sensitivity and specificity, it has advantages over the microscopic test of not requiring a microscope or judgment of a skilled technologist with results available in about 15 minutes. Additionally, it can reveal antigen when cultures are negative caused by prior antimicrobial treatment or the result of contamination after transport to a laboratory.

A new diagnostic modality is the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, which shows distinctive protein profiles for each bacterial species and the three biotypes of *Y. pestis*.[38] Its advantages are lack of requirement for expensive antibodies and PCR reagents, along with speed and automated ease of use. Another novel technique is a PCR/electrospray ionization-mass spectrometry for revealing DNA signatures characteristic of *Y. pestis*.[10] It has been used to screen environmental and clinical samples in settings where bioterrorism pathogens are suspected.

TREATMENT

The antibiotic of choice for plague during the last 50 years of the 20th century was streptomycin. Alternatives were tetracycline, chloramphenicol, or combinations with one another, sometimes with added sulfonamide. With discontinuation by manufacturers of streptomycin and chloramphenicol in most countries because of toxicities, there was a need to test newer drugs. In a randomized controlled comparison of gentamicin and doxycycline in 65 patients in Tanzania, both drugs were highly effective.[14] Because of streptomycin being the previous drug of choice, gentamicin can take its place with assurance that, by belonging to the same aminoglycoside class as streptomycin, it will perform optimally. On the other hand, doxycycline appears equally effective with advantages over gentamicin of oral dosing, lack of nephrotoxicity, and no need to monitor blood concentrations. The fluoroquinolone ciprofloxacin was effective in protecting mice against lung infection after exposure by inhalation to aerosols of *Y. pestis*.[39,40] It is recommended for prophylaxis after exposure to suspected cases and as an alternative drug for infected patients.[15,16,20] However, fluoroquinolones have not been adequately evaluated in human treatment trials and should not be chosen as a first-line drug. As a rapidly fatal disease advancing to septic shock and multiorgan failure, optimal care sometimes requires an intensive care unit for support of the circulation and for mechanical ventilation.

Antibiotic choices have not been disrupted by emergence of resistant strains, as shown by a survey of 392 strains collected from 17 countries.[41] All remained susceptible to antimicrobials currently recommended for treatment, including streptomycin, gentamicin, tetracycline, doxycycline, trimethoprim-sulfamethoxazole, and ciprofloxacin. Although *Y. pestis* can accept resistance plasmids from other bacteria under laboratory conditions, it has rarely been done in nature, probably because its habitat in the flea gut and normally sterile animal tissues does not bring it into contact with large populations of other bacteria affected by selective pressures of antibiotic usage.

ANIMALS AND VECTORS

Plague as a zoonosis has entrenched reservoirs in various rodent species on the continents of North and South America, Africa, and Asia. In most endemic regions that have been surveyed by mammalogists, there is an enzootic species that acquires infection by flea bites but is resistant to becoming ill or dying caused by an effective immune response, thus allowing prolonged bacteremia with opportunity for flea-borne transmission.[42] The enzootic species vary by geography. For example, it is black rats and shrews in Madagascar, great gerbils in Kazakhstan, jirds in Algeria, marmots in China, and ground squirrels, deer mice, and voles in the United States.[7,42–44] Infection is sometimes transferred by shared fleas to less resistant peridomestic rodents, like rats, shrews, and prairie dogs, which can propagate epizootics with die-offs and transmission to humans. Other animals, such as rabbits, field mice, squirrels, camels, and carnivores, including cats and dogs, become infected through flea bites or ingestion of rodents, but these animals are considered peripheral to the maintenance of infection in the more stable reservoir species. Maintenance of infection during inter-epidemic periods has been explained by the ability of *Y. pestis* to survive for prolonged periods in the soil, from which burrowing rodents might directly acquire the bacteria.[45,46] Pet dogs in the United States seem to have an indirect role by carrying rodent fleas into the home, as demonstrated in a case-control study showing that sleeping in a bed with a pet dog was a risk factor for human plague.[23]

Most human infection is caused by the bite of an infected rodent flea. The classic vector is the Oriental rat flea *Xenopsylla cheopis*, but other flea species, such as *Oropsylla montanus*, the primary vector on rock squirrels and ground squirrels for human plague in the United States, also can transmit plague.[17,42] A widely held view about flea transmission was that the most efficiently transmitting flea *X. cheopis* became blocked in its upper gut after a blood meal caused by clots formed by coagulase produced by *Y. pestis*, resulting in repeated feeding attempts with regurgitation of bacteria into a host. This idea was challenged by experiments showing that unblocked *Oropsylla* fleas were better transmitters because they became infectious within a day of feeding and remained infectious for at least 4 days, whereas flea blockage takes about 2 weeks to develop and reduces fleas' survival times.[17] Other blood-sucking insects are not implicated, but research with body lice in a rabbit model of plague showed that lice could transmit infection, thus providing evidence for alternative vectors that may have been significant in medieval plague epidemics when transmission by rodent fleas did not fit with historic descriptions.[47]

VACCINES

Older plague vaccines, killed whole cell vaccines, and the live attenuated nonpigmented mutant vaccine EV76, were developed more than 100 years ago and administered to millions of people. They are rarely used today because of

toxicities, limited evidence for efficacy to prevent plague, and limited commercial availability. In the past decade, efforts to develop and test new vaccines for bioterrorist exposure have focused on recombinant subunits of the F1 capsule and the V outer membrane of *Y. pestis*. Both subunits are plasmid-encoded virulence factors that generate protective antibodies during natural infection. F1 is the antiphagocytic protein capsule made by the caf1 gene on the pFra plasmid and V antigen is an outer protein encoded by the pYV plasmid that is part of the needle-like injectisome of a type III secretion system used by bacteria to inject Yops into host cells for disruption of phagocytosis and diminishing the inflammatory response. Two recombinant vaccines have been tested in Phase I and Phase II human studies of safety and immunogenicity given as priming injections followed by booster injections 21 days later. One vaccine contained a defined amount of the subunits, designated rF1 and rV, and the other was a fusion protein, designated rF1-rV. The rF1 and rV vaccine was given to 24 healthy volunteers, whereas eight volunteers received a placebo of the adjuvant alhydrogel. No serious vaccine-related adverse events were reported. In a larger trial, 600 healthy persons received either of the two vaccine preparations to determine which preparation and dose was optimal as a marketable product. Antibodies against both F1 and V were elicited and shown to be protective against plague pneumonia when transferred passively to mice before inhalational challenge with virulent *Y. pestis*.[18] Because cell-mediated immunity in addition to an antibody response is required for protection against plague pneumonia, the rF1 and rV vaccine was given to mice, which were tested for recall response of their lymphocytes. These experiments indicated that the vaccine also induced cell-mediated responses.[19] It has been suggested that both helper T lymphocyte responses of the Th1 type that govern immune cellular interactions and Th2 responses that give rise to antibody production are needed for optimal vaccine protection as a result of the concept in plague that early intracellular bacterial growth in macrophages, where bacteria survive by inhibiting nitric oxide in phagosomes and when a Th1 response would be relevant, and a subsequent extracellular growth of bacteria, when the Th2 response would drive antibody-dependent defense, are both essential for plague pathogenesis.[48,49] It is anticipated that a vaccine will be available in the next decade and will be useful for military exposure on a battlefield, first responders to bioterrorist use, laboratory workers, and travelers to endemic regions of the world.

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